

### **Specification Amendments**

After Paragraph 15, please add a new paragraph to the specification as follows:

In addition, any member of a group consisting of hydronium, lithium, sodium, potassium, tetramethylammonium, tetraethylammonium, tetrapropylammonium, tetrabutylammonium, benzyltrimethylammonium, benzyltriethylammonium, benzyltripropylammonium, benzyltributylammonium, alkoxybenzyltrimethylammonium ions can be used as a non-hydrolyzing cation for a strong or weak electrolyte auxiliary agent, and any member of a group consisting of hydroxide, chloride, bromide, iodide, sulfate, nitrate, methanesulfonate, ethanesulfonate, benzenesulfonate, toluenesulfonate, naphthalenesulfonate, benzenedisulfonate, naphthalenedisulfonate and alkoxybenzenesulfonate ions can be used as a non-hydrolyzing anion for a strong or weak electrolyte auxiliary agent. Further, any member of a group consisting of ammonium, monoalkylammonium, dialkylammonium, trialkylammonium, arylalkylammonium, alkoxyarylalkylammonium ions can be used as a hydrolyzing cation for a weak electrolyte auxiliary agent, and any member of a group consisting of alkylcarboxylate, arylcarboxylate, alkylarylcarboxylate, alkoxyarylcarboxylate, phenolate and alkoxyphenolate ions can be used as a hydrolyzing anion for a weak electrolyte auxiliary agent.

Please amend Paragraphs 33, 34, 37, 38, and 43 of the specification as follows:

[0033] FIG. 3 shows a typical pH gradient created during isoelectric focusing separation of a mixture containing only carrier ampholytes, ampholytic sample components, an added anodic ampholytic auxiliary agent, and an added cathodic ampholytic auxiliary agent, but

no salts, in a capillary isoelectric focusing system comprising an anode compartment, an anodic auxiliary compartment, a separation capillary, a cathodic auxiliary compartment, and a cathode compartment. FIG. 3 illustrates the effect of adding two auxiliary compartments, 38 and 46, to the isoelectric focusing apparatus. In this example, the auxiliary compartments are created by tubes attached to the separation capillary, 42, which extends between the ends 40 and 44 of the respective tubes forming the auxiliary compartments 38 and 46, such that fluid can flow through elements 38, 42, and 46. The volume of the separation capillary is  $V_{sep}$ . In this example, the diameter of the attached tubes is larger than the diameter of the separation capillary. The auxiliary compartments are separated from the electrode compartments by membranes, line 36 and 48. Prior to isoelectric focusing, the auxiliary compartments and the separation capillary are filled with the carrier ampholytes, the sample components, and the auxiliary agents.

[0034] After isoelectric focusing separation, the anodic auxiliary agent is present in the auxiliary compartment, 38, closest to the anode with a volume of  $V_{auxanode}$ , the cathodic auxiliary agent is present in the auxiliary compartment, 46, closest to cathode with a volume of  $V_{auxcathode}$ , and the carrier ampholytes and the sample are present in the separation capillary. Similarly to the embodiment of Fig. 1, the pH increases approximately linearly across the length of the separation capillary, 42, shown by line 33.

[0037] FIG. 5 shows a pH gradient created during isoelectric focusing separation of a mixture containing carrier ampholytes, ampholytic sample components, an added anodic ampholytic auxiliary agent, an added cathodic ampholytic auxiliary agent, and salts, in a capillary isoelectric focusing system comprising an anode compartment, an anodic auxiliary compartment, a separation capillary, a cathodic auxiliary compartment, and a cathode compartment. FIG. 5 shows that by adjusting the amount of the auxiliary agents used, one can compensate for the presence of salt in the sample. In FIG. 5, the salt concentration of the sample is the same as in FIG. 4, but the amount of auxiliary agents added to the sample has been reduced to such an extent that after isoelectric focusing, the auxiliary agents only occupy sections 60 and 64 of the auxiliary compartments. Because only the carrier ampholytes and the sample components are now in the separation capillary, 62, the pH gradient, which was compressed in Fig. 4, now extends the length of the separation capillary 62, as shown by line 66. ~~The~~ the electropherogram will be similar to what was obtained in FIG. 3, i.e., the detrimental changes caused by the presence of salt in the sample will have been eliminated.

[0038] The auxiliary agents added can absorb light at the wavelength of detection, can fluoresce, or can be transparent, but there is an advantage in iCIEF to using ultraviolet absorbing agents, because of the ease of determining the appropriate amounts of auxiliary agent needed in the sample. As soon as the boundaries of the added UV absorbing auxiliary agents are observed, a sufficient amount of auxiliary agent has been added. If no boundaries are observed then insufficient amounts of auxiliary agents have been added. If the boundaries penetrate too far into the capillary, the amount of auxiliary agent added is too high and needs to be reduced.

[0043] FIGS. 9-12 show the use of anodic and cathodic auxiliary agents to eliminate

compression of the pH gradient that was caused by the presence of salt in the sample. The sample is a mixture of pI markers DNS-Asp, DNS-Phe, ~~DNS-Trp~~ DNS-GABA, terbutaline and tyramine, dissolved in 8% pH 3-10 Ampholine carrier ampholytes.